



Figure 4. The complete DNA sequence of the coding region of *Naegleria* gene TTK (including the TAA stop codon). The segment of the gene that encodes thiaminase I is underlined.

(SEQ ID NO. 1)

ATGTCCACTCAACCAAGACACTCACTGTTGGTCTCTTCCCATATCTTCCTTCTTGGAAATGAAAATGGCAACGAAGTTAA
ATTGATCAATTTGATCAAGGATGTTTTGCCAACTCAGGTTTTCCGGATATAATATCGAATATACCGAATTTGATTGTTACA
GTGATGCTAGTCTTCAAAGTCTTCCAGATGTTTTCTCAACTGATAGCATTTCCTTCCATATCTTGTTTCTTTGGGTGGT
GTCAAGAGTTTGGATGAATCATTGGTTCGTGGTGTACTGGTGATTGTCATAGTTTGTTCCTCAAGTGCCTCTGTCAA
TGGTTCGGTTTATGGTTTCCACAATACTTGTGCTCAAACCTTTTATTGTCTCACCAAATGGTACTCAACAAGCATCTT
CCCTTTAGAATTGGCTCAAAAGGTTGGTTATGAACAAATTGTTTATCCAGATGTTGCCTCTTCTAGTTCTTTCACAGTT
TTCGGATTGTATCAACAATTACTCCAATCATCATCATCAGCTGCAGTTGATATCAAGGCCCTCGATCTTCCACAATCTGG
TGACCAAGTCAACAAGGATATCACTCAAAAATATAGAACCATTTTGGATTCAACAGTTGTTGCCTCTCAAGAGAAATATA
TTAACTCTGTAAAGCAAGGTAAACCAATTTCAAACCTACTATGTCGGATATAGTGAAAAGTATGTGTGAAAATTAAGGATATC
ATCAGAGATCAACAATACAATGTTCAACTCATTGGTACCTCTGATAAGCCATACGTTTATACTGATGTTTTGGCTTTGAA
TTCCAATTTGTGTGATGAAAAGCAAAAGGTTGCTGTTGAAGTTATCAAGAATTTATTGACTAATACTTTAGTTTTGGACT
TGTTGGGTCTCGGATTAACCTCTCCAGCCAACAAGAATGGTATTGCTCATTTGGCTAAATCATCAAACCTTTTATGCTCAA
TTGAGCCAACAATTCGATGCCAAGGAAAGTGAAGTTAGAGTTTTGAGATGTGTGACTTTGCTAACAAGGAAGTTAAGAA
TTGTGCTGGTGTCTTGAGACCATTCTTCAACATATTGCTGTGTGCTACTTTGCGTTGTTGACTGCTGACACTGTGCGAAA
AGGCTAAGAGTGGTCACCTGGTATGCCAATTGGTATGTACCAATTGCCTATGTTTTGTGGAAGTCTTCTTCAAATCA
TCTAAGGATGATGTCAATTGGTTGAACAGAGATAGATTTGTTTTGAGTAATGGTCACGGTTGTACATTGCTTTATGCCAT
GTTGCACCTCACTGATTGTAACCTTGAGTTTGGATGATCTCAAGAATTTCAAGAAGTTGCATTCCAAGACTCCTGGTCACC
CAGAATATGGTCACACTGAAGGTGTTGATGCTACTACTGGTCCATTGGGTCAAGGTGTTTGTAATGCTATTGGTATGGCT
CTCTCTGAAGCTCACTTGGCTGCTCGTTTTCAATAAGGATGGACAAAATATCTTTGATCACCACACCTATGTTTTCTTGG
TGATGGTTGTTTATGGAACGTGTTGCTATGGAAGGTCTCTCATTTGCTGGTCACCAAAAGTTGAACAAGTTGATTGTTT
TCTATGATGACAATAGTATTACTATTGATGGTAAGACTGAATTGACCTTTACTCAAAATACTCCAGAAAGTCATGAGAGGT
TTTGGATGGCACGTAATTGTTGTCGACAAGGCTGATAATGACTTGGTTGGTATTAAGGAAGCTATTTTGAAGCTCACAC
TGTTACTGACAAGCCAATCATGATCGTTTGAAGACTACAATTGGTTATTCCTCAAAGGTTCAAGGTACTGCTAAGGTTT
ACGTTTCTCCATTGGGTGCTGATGGATTGAAGAATTTGAAGGAACTTGTGGTTTCACTGGTAATGATTTCTTCCATGTT
CCAGAAATTTGTCAGAAAGGACTTTGCTACTGTCAATTAATAGAAATAGTGAAGGCTCTCTCAATGGAAGCAAGTTAAATC
TGCCTATGATACCACTCATGCTACTGAATCCCAACTCCTCCAAAGAATGATTAATCACGAATTGGAAGGTGATGTTATGG
AAAAGTTGCCAAAATACCTCGAACAAAAGAAGATTGCTACCAGATCTACATCTCAACAAGTTTGAATGCCATCTATCCA
CTCATTCCCTTCTCTCGTTGGTGGTTACAGTGACTTGACTCCATCCAACCTTGACTGATGTAACCTGGATGTCAAGATTTCCA
ACCAAACAATAGAGTTGGTAGATATATCAGATTTGGTGTCCGTGAACATGCCATGGTTGCTATTGCCAATGGTATTCTCT
ATCATGGTGTCTTAGAACCTATGTTGGTACATTCTTGAACCTTGCTTCATATGCTTTGGGTGCTATCAGATTGAGTGCC
TTGTCTGGTCTTCCAAATATTTATGTTTTCACTCATGACAGTATTGGTCTTGGTCAAGATGGTCCAACTCACCAACCTGT
TGAAGTTTACCAATGTTGATAGCCATTCCAAATCACATTGTTTTCAGACCTGCTGATGGTAGAGAAACCAGTGGTGCTT
ATTTGTGGGCTGTTCAATCAAAGAAGACTCCATCCTCAATGATTCTTCTCGTCAAGATTTGCCACAATTGACTGGTACT
GATATTTCAAAGGTTGCTTTGGGTGCCTATGTTATCCAAGGTGATGCTACTCCTGATGTTGTCCTTGTGGTACTGGTTC
TGAAGTTTCCCTCATGGTTGAAGCTGCTGAAAAGTTGAAGGCTAACCTTAAGGTTAACGTTGTTTCCATGCCAAGTTGGG
AATTGTTTGTTCGTCAATCAGAAGAATACAGGAAGACTGCTTCCAGATGGTATTCCAGTTGTGAGTGCCGAAGCTTCA
TCAACCTTTGGTTGGACAAGCTTTGCTCACTATGCTGTTGGTATGACTACTTTCGGTGCTAGTGCTGCTGCTGAAGAAGT
TTACAAACTCCTCAAGATTACCTCAGACAATGTTGCTGAAAAGGCCACCAAAATTGGTTACCAAGTATGGTAAGCAAGCTC
CAAGACTCAGCTTGTCTCTTGTGGTGAAGAAGCTCTAA



Figure 5. The amino acid sequence encoded by the entire *Naegleria* TTK gene

(SEQ ID NO. 2)

MSTQPKTLTVGLFPYLP SWNENGNEVKLINLIKDVLPQTQVSGYNIEYTEFDCYSDASLQSLPDVFSTDSIFLPYLVSLGG
VKSLDESLVRGVTGDLHSFVSSSASVNGSVYGFPPQYLCNFFLLSSPNGTQQASSLLELAQKVGYEQIVYPDVASSSSFTV
FGLYQQLLQSSSSAAVDIKASDLPQSGDQVNKDITQKYRTILDSTVVASQREYINSVKQKPI SNYVVGYS ESMCEIKDI
IRDQQYNVQLIGTSDKPYVYTDVLALNSNLCDEKQKVAVEVIKNLLTNTLVLDLLGLGLTLPANKNGIAHLAKSSNFY AQ
LSQQFDAKESEVRVLR CVD FANKEVKNCAGVLRPFLOHIAVATLRCLTADTVEKAKSGHPGMPIGMSPIAYVLWKFFFKS
SKDDVNWLNDRFVLSNGHGCTLLYAMLHLTDCNLSLDDLKNFRSLHSKTPGHPEYGHTEGVDATTGPLGQGV CNAIGMA
LSEAHLAARFNKDGQNI FDHHTYVFLGDGCLMERVAMEGLSFAGHQKLNKLIVFYDDNSITIDGKTELFTQNTPEVMRG
FGWHVIVVDKADNDLVGIKEAILEAHTVTDKPIMIVCKTTIGYSSKVQGTAKVHGSP LGADGLKNLKETCGFTGNDFFHV
PEIVRKDFATVINRNSEKLSQWKQVKSAYDTTHATESQLLQRMINHELEGDVMEKLPKYLEQKKIATRSTSQQVLNAIYP
LIPSLVGG SADLTPSNLT DVTG CQDFQPNNRVGRYIRFGVREHAMVAIANGILYHGVLR TYVGTFLNFASYALGAIRLSA
LSGLPN IYVFTHDSIGLGQDGPTHQPVEVLPMLIAIPNHIVFRPADGRETS GAYLWAVQSKKTPSSMILSRQDLPQLTGT
DISKVALGAYVIQGDATPDVVLVGTGSEVSLMVEAAEKLKANLKVNVVSMPSWELFVRQSE EYRKTVPDGI PVVSAEAS
STFGWTSFAHYAVGMTTFGASAAAEVYKLLKITS DNVAEKATKLVTKYGKQAPRLSLSLVGEEL

Figure 6. The DNA sequence of the 1068 bp *Naegleria* thiaminase I segment, as cloned in pNB1+ and expressed from the 3414 base genomic sequence of gene TTK.

(SEQ ID NO. 3)

ATGTCCACTCAACCAAAGACACTCACTGTTGGTCTCTTCCCATATCTTCCTTCTTGGAAATGAAAATGGCAACGAAGTTAA
ATTGATCAATTTGATCAAGGATGTTTTGCCAACTCAGGTTTCCGGATATAATATCGAATATACCGAATTGATTGTTACA
GTGATGCTAGTCTTCAAAGTCTTCCAGATGTTTTCTCAACTGATAGCATTTTCCTTCCATATCTTGTTTCTTTGGGTGGT
GTCAAGAGTTTGGATGAATCATTGTTTCGGTGTACTGGTGATTTGCATAGTTTGTTCCTCAAGTGCCTCTGTCAA
TGGTTCCGTTTATGGTTTCCACAACTACTTGTGCTCAAACCTTTTTATTGTCCTCACCAAATGGTACTCAACAAGCATCTT
CCCTTTTAGAATTGGCTCAAAAGGTTGGTTATGAACAAATTGTTTATCCAGATGTTGCCTCTTCTAGTTCTTTCACAGTT
TTCGGATTGTATCAACAATTACTCCAATCATCATCATCAGCTGCAGTTGATATCAAGGCCTCTGATCTTCCACAATCTGG
TGACCAAGTCAACAAGGATATCACTCAAAAATATAGAACCATTTTGGATTCAACAGTTGTTGCCTCTCAAAGAGAATATA
TTAACTCTGTAAAGCAAGGTAAACCAATTTCAAACTACTATGTCGGATATAGTGAAAGTATGTGTGAAATTAAGGATATC
ATCAGAGATCAACAATACAATGTTCAACTCATTGGTACCTCTGATAAGCCATACGTTTATACTGATGTTTGGCTTTGAA
TTCCAATTTGTGTGATGAAAAGCAAAAGGTTGCTGTTGAAGTTATCAAGAATTTATTGACTAATACTTTAGTTTTGGACT
TGTTGGGTCTCGGATTAACTCTCCAGCCAACAAGAATGGTATTGCTCATTGGCTAAATCATCAAACCTTTTATGCTCAA
TTGAGCCAACAATTCGATGCCAAGGAAAGTGAAGTTAGAGTTTTGAGATGTGTTGACTTTGCTAACAAGGAAGTTAAGAA
TTGTGCTGGTGTCTTGAGACCATTTCCTT

Figure 7. The encoded amino acid sequence of the expressed *Naegleria* thiaminase I gene, along with its codons, and with residues numbered (nucleotide/amino acid).

(SEQ ID NO. 3 / SEQ ID NO. 4)

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1/1                      31/11
ATG TCC ACT CAA CCA AAG ACA CTC ACT GTT GGT CTC TTC CCA TAT CTT CCT TCT TGG AAT
Met ser thr gln pro lys thr leu thr val gly leu phe pro tyr leu pro ser trp asn

61/21                      91/31
GAA AAT GGC AAC GAA GTT AAA TTG ATC AAT TTG ATC AAG GAT GTT TTG CCA ACT CAG GTT
glu asn gly asn glu val lys leu ile asn leu ile lys asp val leu pro thr gln val

121/41                      151/51
TCC GGA TAT AAT ATC GAA TAT ACC GAA TTT GAT TGT TAC AGT GAT GCT AGT CTT CAA AGT
ser gly tyr asn ile glu tyr thr glu phe asp cys tyr ser asp ala ser leu gln ser

181/61                      211/71
CTT CCA GAT GTT TTC TCA ACT GAT AGC ATT TTC CTT CCA TAT CTT GTT TCT TTG GGT GGT
leu pro asp val phe ser thr asp ser ile phe leu pro tyr leu val ser leu gly gly

241/81                      271/91
GTC AAG AGT TTG GAT GAA TCA TTG GTT CGT GGT GTT ACT GGT GAT TTG CAT AGT TTT GTT
val lys ser leu asp glu ser leu val arg gly val thr gly asp leu his ser phe val

301/101                      331/111
TCC TCA AGT GCC TCT GTC AAT GGT TCC GTT TAT GGT TTC CCA CAA TAC TTG TGC TCA AAC
ser ser ser ala ser val asn gly ser val tyr gly phe pro gln tyr leu cys ser asn

361/121                      391/131
TTT TTA TTG TCC TCA CCA AAT GGT ACT CAA CAA GCA TCT TCC CTT TTA GAA TTG GCT CAA
phe leu leu ser ser pro asn gly thr gln gln ala ser ser leu leu glu leu ala gln

421/141                      451/151
AAG GTT GGT TAT GAA CAA ATT GTT TAT CCA GAT GTT GCC TCT TCT AGT TCT TTC ACA GTT
lys val gly tyr glu gln ile val tyr pro asp val ala ser ser ser ser phe thr val

481/161                      511/171
TTC GGA TTG TAT CAA CAA TTA CTC CAA TCA TCA TCA TCA GCT GCA GTT GAT ATC AAG GCC
phe gly leu tyr gln gln leu leu gln ser ser ser ser ala ala val asp ile lys ala

541/181                      571/191
TCT GAT CTT CCA CAA TCT GGT GAC CAA GTC AAC AAG GAT ATC ACT CAA AAA TAT AGA ACC
ser asp leu pro gln ser gly asp gln val asn lys asp ile thr gln lys tyr arg thr

601/201                      631/211
ATT TTG GAT TCA ACA GTT GTT GCC TCT CAA AGA GAA TAT ATT AAC TCT GTA AAG CAA GGT
ile leu asp ser thr val val ala ser gln arg glu tyr ile asn ser val lys gln gly

661/221                      691/231
AAA CCA ATT TCA AAC TAC TAT GTC GGA TAT AGT GAA AGT ATG TGT GAA ATT AAG GAT ATC
lys pro ile ser asn tyr tyr val gly tyr ser glu ser met cys glu ile lys asp ile

721/241                      751/251
ATC AGA GAT CAA CAA TAC AAT GTT CAA CTC ATT GGT ACC TCT GAT AAG CCA TAC GTT TAT
ile arg asp gln gln tyr asn val gln leu ile gly thr ser asp lys pro tyr val tyr

781/261                      811/271
ACT GAT GTT TTG GCT TTG AAT TCC AAT TTG TGT GAT GAA AAG CAA AAG GTT GCT GTT GAA
thr asp val leu ala leu asn ser asn leu cys asp glu lys gln lys val ala val glu

841/281                      871/291
GTT ATC AAG AAT TTA TTG ACT AAT ACT TTA GTT TTG GAC TTG TTG GGT CTC GGA TTA ACT
val ile lys asn leu leu thr asn thr leu val leu asp leu leu gly leu gly leu thr l

901/301                      931/311
CTC CCA GCC AAC AAG AAT GGT ATT GCT CAT TTG GCT AAA TCA TCA AAC TTT TAT GCT CAA
leu pro ala asn lys asn gly ile ala his leu ala lys ser ser asn phe tyr ala gln

961/321                      991/331
TTG AGC CAA CAA TTC GAT GCC AAG GAA AGT GAA GTT AGA GTT TTG AGA TGT GTT GAC TTT
leu ser gln gln phe asp ala lys glu ser glu val arg val leu arg cys val asp phe

1021/341                      1051/351
GCT AAC AAG GAA GTT AAG AAT TGT GCT GGT GTC TTG AGA CCA TTC CTT
ala asn lys glu val lys asn cys ala gly val leu arg pro phe leu

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Abbrev.	Genbank	Enzyme and Organism
ScTKT1ct -	P23254	Transketolase of <i>Saccharomyces cerevisiae</i>
CpTKT7ct -	Q42677	Transketolase of <i>Craterostigma plantagineum</i>
EcTKT2ct -	P33570	Transketolase of <i>Escherichia coli</i>
BsTKTct -	P45694	Transketolase of <i>Bacillus subtilis</i>
MgTKT -	P47312	Transketolase of <i>Mycoplasma genitalium</i>
MjPTK1 -	Q58092	Transketolase of <i>Methanococcus jannaschii</i>
BSTP -	P45741	Thiaminase I precursor from <i>Bacillus thiaminolyticus</i>
N4OKAT -	-----	Thiaminase I of <i>Naegleria gruberi</i> , aa 1-356

(SEQ ID NO.12) Peptide A -> ASDLPQSGD-QVNK

Figure 8, continued.

ScTKT1ct	RSLPNIQVWRPADGN-EVSAAYKNSLESKHTPSIIALSQRNLPQLEGS---SIESASKGG	278
CpTKT7ct	RAMPNILTLPADGN-ETAGAYRAAVQNGERPSILVLARQKLPQLPGT---SIEGVSKGG	
EcTKT2ct	RLTPNFSTWRPCDQV-EAAVGWKLAVHRNGPTALILSRQNLAQVERTPD-QVKEIARGG	
BsTKTct	RAMPNLSLIRPADGN-ETAAAWKLAVQSTDHPTALVLTRQNLPITDQTSEEALAGVEKGA	
MgTKT	RAIENVCVFRPCDEK-ETCAGFNYGLLSQDQTTVLVLTRQPLKSIDNTD--SLKTL-KGG	
MjPTK1	RAIPNMVVIAPTDYY-HTKNVIRTIAEYKG-PVYVRMPRRDTEIIYENEEATFEIGKKG	
BsTP	RLLINMAGEKPSQYVPEDGDAYVRASWFAQ-GSGRAFIGYSESMMRMG--DYAEQVRFK	
N40KAT	DITQKYRTILDSTVV-ASQREYINSVKQKG-PISNYVVGYSMCEIK--DIIRDQQYN	

Peptide B -> TILDSTVV-ASQR (SEQ ID NO. 13)

ScTKT1ct	YVLQDVAN-----PDIIILVATGSEVSLSVEAAKTLAAKNIKARVVSLPDFFTFDKQPLE-	332
CpTKT7ct	YVISDNISRGNSKPDVILIGTGSELEIAARAGDELRKEGKKVRVSVLCWELFAEQSEK-	
EcTKT2ct	YVLKDSGG---KPDIIILVATGSEMEITLQAAEKLAGEGRNVRVSVLPSTDFDAQDEE-	
BsTKTct	YVVSXSKN--E-TPDALLIASGSEVGLAIEAQAEELAKENIDVSVVSMPSMDRFKQSD-	
MgTKT	YILLDRKQ-----PDLIIAASGSEVQLAIEFEKVLTKQNVKVRILSVPNITLLLKQDEK-	
MjPTK1	ILVDG-----EDLTIATGEEVPEALRAGEILKENGISAEIVEMATIKPIDEEIKK	
BsTP	PISSSAG-----QDIPLFYSDVSVNSKTAHPELAKKLANVMASADTVEQALRPQADGQ	
N40KAT	VQLIGTS-----DKPYVYTDLALNSN---LCDEKQKVAVEVIKNLLTNTLVLDLLG-	

ScTKT1ct	YRLSVLPDNPVI-MSVEVLATTCWGKYAH-----QSFGIDRFGASGKAPEVFKFFGFTP	385
CpTKT7ct	YRETVLPSTGVTARVSVEAGSTFGWERFIGP-KG--KAVGIDRFGASAPAEFLFKEFGITV	
EcTKT2ct	YRESVLPNVAARVAVEAGIADYWKYVGL-KG--AIVGMTGYGESAPADKLFPFFGFTA	
BsTKTct	YKNEVLPAADVKKRLAIEMGSSFGWGKYTGL-EG--DVLGIDRFGASAPGETIINEYGFVS	
MgTKT	YLKSLFDANSSL-ITIEASSSYEWFCFKKY-VKNHAHLGAFSFGESDDGDQVYQQGFNL	
MjPTK1	SKDFVVTVEDHSIIGGLGGAVEIASNGLNKKLLRIGINDVFRSGKADELLKYYGLDG	
BsTP	YPQYLLPARHQV-YEALMQDYPIYSELAQIVNK--PSNRVFRLGPEVRT-WLKDAKQVLP	
N40KAT	-LGLTLPANKNG-IAHLAKSSNFYAQLSQ-----QFDAKESEVRVLRVDFANKEV	

Peptide C -> SSNFYAQLSQ-----QFDAK (SEQ ID NO. 14)

ScTKT1ct	EGVAERAQKTIAFYKGDKLISPLKKAF	412	(SEQ ID NO. 5)
CpTKT7ct	EAVVA-AAKEIC-----		(SEQ ID NO. 6)
EcTKT2ct	ENIVAKAHKVLGVKGA-----		(SEQ ID NO. 7)
BsTKTct	PNVVNRVKALINK-----		(SEQ ID NO. 8)
MgTKT	ERLMKIFTSLRN-----		(SEQ ID NO. 9)
MjPTK1	ESIAKRIMEEMKKE-----		(SEQ ID NO. 10)
BsTP	EALGLTDVSSLAS-----		(SEQ ID NO. 11)
N40KAT	KNCAGVLRPFL-----		(SEQ ID NO. 4)